1B UPLAND RICE

1B.1 Collaborative Project between CIAT and Cirad Rice improvement through the use of synthetic populations

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Abstract

The Cirad/CIAT rice collaborative project focuses on the development and enhancement of rice synthetic populations through recurrent selection (RS) with the goal to develop and diffuse improved material, populations or lines, for various rainfed ecosystems in Latin America and the Caribbean (LAC). Our breeding strategy is based on the development of broad-base populations, their improvement thought recurrent cycles of selection and recombination, and the exploitation of their genetic wealth for line development through conventional breeding.

Within our network of rice breeders from LAC, we released improved material as part of the breeding activities for upland rice in Colombia and shared germplasm of interest to LAC collaborators as part of pre-breeding activities.
We are developing large scale phenotyping methods adapted to field conditions to evaluate response to drought among synthetic populations. The thermographic infra-red technology was assessed for screening large number of families, as well as for single-plant evaluation within large populations. This evaluation method allowed us to identify interesting progenitors to develop new population with increased resilience to drought. Allelic variability measured in several synthetic populations revealed high level of neutral diversity.
These activities are part of a much broader mission on the management of synthetic populations for more efficient use of RS breeding and for the development of a marker assisted recurrent selection program.

Background

Recurrent selection (RS) method of breeding was designed to gradually increase the frequency of desirable alleles and allele combinations, while maintaining genetic variability for future selections in a genetically broad-based population. RS is a cyclical method of population improvement that consists in successive cycles of evaluation, selection and intermating. RS method is complementary to conventional breeding by which the variability of the population is exploited for variety development. RS has been used in plant species to enhance yield, alter seed or plant quality, increase pest resistance, and improve tolerance to the environment (Hallauer, 1985).

RS breeding was developed and has been used by Cirad rice breeders for almost two decades. Since 1996, the Cirad/CIAT rice project has worked on developing and enhancing rice germplasm, through RS and conventional breeding, with the aim to deliver genetic resources, training and networking with partners of the public and private research sectors in Latin America and Caribbean (LAC) (Châtel and Guimarães, 1997).

Among the panel of agronomic and adaptive traits considered by rice breeders, we chose to concentrate on resilience to water deficit. Drought stress is the most severe abiotic constraint in upland rice (Bernier et al., 2009) and a serious limit to crop production in LAC (AFP, 2009; FAO, 2005). This important trait is of concern to various rice growing areas reported with actual or future severe drought events. Furthermore, in the context of eco-efficient agriculture, water use efficiency is a critical characteristic to look for among breeding material. However, drought phenotyping can be a limiting factor when sizeable experiments are conducted. Phenotyping methods must follow the same trends as the advances in biotechnology in order to reduce the gap between genotyping and phenotyping capacity and to increase the discovery of true marker-trait associations.

Molecular makers are important tools for population improvement (Courtois et al., 2005; Ramis et al., 2005). They contribute to assist selection by using markers tightly linked to the gene of interest as screening tool. Furthermore, molecular markers are means to monitor diversity within populations. Synthetic populations being considered as a source of allelic richness, it is essential to insure high level of genetic diversity and avoid genetic erosion. Moreover, with targeted markers, populations can be evaluated for their functional diversity, to assess their content in particular allelic variants, and to monitor maintenance of various genes of interest within the populations.

Through the development and improvement of rice synthetic populations our goal is to respond to the need of broadening the genetic base of the material used in breeding programs, and to develop diverse and enhanced germplasm with given traits of interest. Our specific objective was to evaluate synthetic populations under drought conditions to, eventually, develop new population targeted for resilience to drought. Molecular markers tools were used to quantify and to characterize the genetic diversity encompassed in synthetic populations and to assess the breadth of their allelic richness.

Material and Methods

One population (PCT-4\0\0\1) was created in 1995 with a large set of progenitors (35 inbred lines). From this original population, four others were generated, one which was advanced for three cycles without selection (PCT-4\0\0\3), two with specific selection pressure and various recombination cycles (PCT-4\SA\8\1; PCT-4\SA\2\1-Bo\4\1), and one with a slightly different initial genetic constitution (PCT-11\0\0\2-Bo\4\1) (Table 1).

Table 1. Rice synthetic populations considered in our study

	Selection cycles	Recombination cycles	Generation
PTC-4\0\0\3	0	3	S0
PTC-4\0\0\3	0	3	S1
PTC-4\SA\8\1	SA (Acid Soils)	8	S1
PTC-4\SA\2\1,Bo\4\1	SA, Bolivia	7	S1
PTC-11\0\0\2,Bo\3\1	Bolivia	5	S1

Eco-physiology and breeding

Large scale phenotyping was set-up during the dry off-season 2008-2009 in the Experimental Station of Santa Rosa (EESR). Evaluation concerned 1000 plants coming from the PCT-4\0\0\3, and 400 S_1 lines coming from all four synthetic populations. Field screening methods consisted in canopy temperature detection by infra-red thermographic camera (NEC IR Camera). Water stress was applied after panicle initiation stage for 15 days. Readings were performed by Alain Audebert (Cirad) under stress condition and under no water limitation. Control of environmental

variation was achieved through the use of soil moisture monitor (Diviner 2000) and proper experimental design as suggested by Myriam Cristina Duque (CIAT).
In the Experimental Station of La Libertad (EELL) the same set of 400 lines was sown during the cropping season for phenotypic evaluation under the natural conditions of the experimental station (savanna type soils; pH 4.8, aluminum toxicity; high disease pressure).

Genetic diversity

Genetic analyses were performed on a random sample of 207 single S_0 plants for population PCT-4\0\0\3. For all four populations, hundred S_1 families per population were selected and analyzed as 10-S_1 plants bulk per family. Allelic diversity was estimated at 18 SSR loci.

Results and Discussion

A. Rice breeding through RS

As member of a rice breeders' network we develop and improve populations in centralized pre-breeding activities. Upland synthetic populations are observed, characterized and improved through RS in our program. Improved lines extracted from this diverse and enriched pool of germplasm are distributed to national programs in the region for further testing.

Germplasm enhancement

A set of 1664 lines at different stage of advancement (S_1 to S_8) were characterized in EELL-2009 and selected to follow the line development activity. Among these, 600 were advanced lines from S_4 to S_8 generation of which 480 were selected for further improvement through conventional breeding.

Population improvement

The four populations were advanced with one cycle of selection during the cropping season at EELL, and one cycle of recombination during the off-season in Palmira (EEP). No specific selection was performed on these populations, only selection for general plant adaptation under natural field conditions and for preserving a wide range of plant types to meet the diverse demand from our partners.

Germplasm release

Yara and Paya, two varieties extracted from the synthetic population PCT-11 were released by our partner from Bolivia - La Paz. These two varieties are specifically adapted to upland hillside small holders in Bolivia.

B. Breeding, eco-physiology and molecular markers

The phenotyping experiment in Santa Rosa (EESR-2008-09) and the results obtained will be described by Alain Audebert in another chapter of this CIAT-Rice project annual report. The outcomes were also reported in the InterDrought III meeting in Shanghai, China (Audebert et al., 2009).

The 400 S_1 families from the four populations were evaluated under drought conditions (EESR-2008-09) and the resulting 400 S_2 families were characterized in La Libertad (EELL-2009) under the natural soil conditions and heavy blast infestation. Seventy five S_1 families were found with low temperature differential between the stress and the control condition, indicating good transpiration potential under water deficit and a certain degree of resilience to drought. Nineteen of these families also showed good agronomic potential in the EELL09 evaluation. This 19 materials present great value traits and could be a group on which to concentrate efforts for line advancement.

Within the synthetic population that was evaluated under water limited conditions, 16 S_0 plants were found with great potential for response to drought. From these selected plants, eight fertile and with good seed set were selected to constitute the pool of progenitors to go through recombination for developing a new population. The recombination cycle occurred in Palmira (EEP) in absence of stress and 240 male sterile plants were harvested in EEP-2009 and pooled to constitute the new population with improved resilience to drought PCT-4\EF\1\3. This population will be further improved through RS to preserve and enhance its genetic variability and its adaptive characteristic.

Molecular markers were used to evaluate the genetic diversity encompassed in a subset of each of the four populations. The results from this study were presented at the symposium Recursos Genéticos para América Latina y el Caribe in Pucón, Chile (Grenier et al., 2009). The source of diversity used to develop the population resulted in high allelic richness. Averaged over the 16 loci, the

genetic diversity, expressed in terms of number of observed alleles per locus (*Na*) and Shannon diversity index (*I*), was high within the populations (Table 2). Across populations, *Na* was greater than 3.133 alleles per locus and the diversity index varied from *I*=0.693 to *I*=0.800.

Table 2. Genetic diversity assessed with the number of observed alleles (Na) and the Shannon diversity index (I)*. Mean and standard error (*SE)* per locus

Population	Na	I
PCT-4\0\0\3	3.133	0.695
	(0.401)	*(0.112)*
PCT-4\SA\8\1	3.200	0.704
	(0.527)	*(0.107)*
PCT-4\SA\2\1-Bo\4\1	3.667	0.800
	(0.607)	*(0.123)*
PCT-11\0\0\2-Bo\4\1	3.400	0.693
	(0.559)	(*0.118*)

* $I = -\sum p_i \ln p_i$

Across loci, *Na* was highly variable from 2 to 10 alleles per locus (Table 3). The three populations trace to specific evolution history, with different selection pressures and different events of recombination. Consequently they showed high level of diversity and significant (p≤0.05) allelic and genotypic differentiations were found between them (Table 3).

Table 3. Number of alleles observed (Na) and test for allelic and genotypic differentiation

		differentiation	
Locus	Na	allelic	genotypic
RM8068	6	***	***
RM6840	3	ns	ns
RM7382	2	***	***
RM5807	3	ns	ns
RM85	3	***	**
RM5608	3	***	***
RM507	2	***	**
RM5907	10	***	***
RM6775	5	***	**
RM5463	10	***	***
RM3394	9	***	***
RM420	2	***	***
RM408	3	***	***
RM477	2	**	**
RM23654	3	*	*
RM7492	4	***	***

ns: no significant; *, **, *** significant at the probability values p≤ 0.05, 0.01 and 0.001 respectively

Rare alleles (≤1%) were present in each population and taking the four populations all together, they represented 34% of the total alleles recoded (Table 4). Furthermore, 11 alleles were unique to any given population. These private alleles were found at various frequencies, and within the PCT-4\SA\2\1,Bo\4\1 four private alleles which were also frequent (>1%) were found.

Table 4. Allelic richness per population and locus. Frequency of observed alelles (Na) / frequency of rare (≤1%) alleles / frequency of "private" alleles

Locus	PCT-4 0\0\3	PCT-4 SA\8\1	PCT-4 SA\2\1-Bo\4\1	PCT-11 0\0\2-Bo\4\1
RM8068	4/0/0	4/1/0	4/0/1*	5/1/0
RM6840	3/0/0	3/0/0	3/0/0	3/0/0
RM7382	2/0/0	2/0/0	2/0/0	2/0/0
RM5807	3/1/1	2/0/0	2/0/0	2/0/0
RM85	2/0/0	2/0/0	3/1/0	3/1/0
RM5608	3/1/1	2/0/0	2/0/0	2/0/0
RM507	2/0/0	2/0/0	2/0/0	2/0/0
RM5907	6/2/0	7/2/0	8/2/1*	6/2/1
RM6775	2/0/0	3/1/0	4/1/1	3/0/0
RM5463	6/0/0	8/0/0	9/1/1*	7/1/1
RM3394	6/1/0	6/1/0	7/0/0	9/2/1*
RM420	2/0/0	2/0/0	2/0/0	2/0/0
RM408	3/1/0	2/0/0	3/0/0	2/0/0
RM477	2/0/0	2/0/0	2/0/0	2/0/0
RM23654	3/0/0	3/1/0	3/0/0	3/0/0
RM7492	2/0/0	2/0/0	3/0/1*	3/0/1*

* Private and frequent (>1%) alleles

Although there was selection pressure (approximately 20%) during the phases of population enhancement for three of the four populations, all four populations maintained high level of genetic diversity, including a significant portion of rare alleles. Because of the different way they were handled, these populations also appear to have fixed some specific and unique allelic variants, probably due to the selective pressure and hitchhiking effect. Functional diversity among these populations will soon be assessed.

Conclusion

RS in rice has proven to be a method with great benefits for rice breeding. Primarily, because the populations initiated more than 14 years ago still maintain high level of genetic diversity, as we could see looking at neutral genetic markers. Furthermore, the populations hold source of favorable alleles for resilience to dry conditions. Frequency of these favorable variants can be increased by population enhancement

through RS, or the favorable allele can be fixed in improved lines through conventional breeding. Besides showing the genetic wealth retained in these synthetic populations, our work presents an advance towards the integration of high-throughput phenotyping and use of molecular markers for improving RS breeding strategy.
Additionally, improved material with specific adaptation to particular ecologies was extracted from the synthetic populations and shared with partners; such as the two most recently released lines targeted for upland hillside small holders in Bolivia.

Future activities

The way forward in rice synthetic population improvement
The next step in our program is to further combine the use of eco-physiology and molecular markers to increase the efficiency of RS breeding. High quality phenotyping and molecular data will be used to study genome wide associations between allelic variants and phenotypes. Breeding scheme will be developed for marker assisted recurrent selection to facilitate selection in a contest of synthetic population improvement.

The monitoring of synthetic populations will be pursed, studying both neutral and functional diversity. Among many other purposes, this will provide an important assessment of the need to introgress other germplasm --exotic lines, improved genetic material, introgression lines, etc…--, in the population to broaden its genetic base; by adding source of allelic variability or new characteristics of interest.

Results and outcomes of these presented research activities conducted by the Cirad/CIAT project will be shared with LAC and others potential partners through the GRUMEGA network.

Additionally, we are fully engaged in a Cirad project “Genetic basis of adaptive diversity in rice; toward tools to pilot selection” for which the diversity in adaptive traits for drought and heat stress will be evaluated among a diverse set of 181 japonica and indica rice. Phenotypic and molecular data will be put together for genome wide association study and to provide rice breeders with parents and molecular markers to conduct marker assisted selection program for these traits. The collection was set-up in a phenotypic trial in Santa Rosa to evaluate response to a 15 days drought period applied at vegetative stage.

Networking

We are hoping to revive the rice breeders' network from LAC (www.grumega.org) through an activity we are launching to characterize the rice growing systems in LAC. This work should lead to a review of the current situation in the region, as well as to set-up observatories to obtain dynamic data base to ultimately monitor the future of rice growing in the region.

Publications

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